

# Putting Metal in the Middle

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Placing metal ions into the center of a porphyrin ring is a significant cellular challenge. [Lundqvist et al. \(2010\)](#) provide snapshots of the AAA+ motor unit of magnesium chelatase, an enzyme that inserts a magnesium ion into the porphyrin ring accompanied by ATP expenditure, in different states of binding to adenosyl nucleotides.

Tetrapyrrole-derived cofactors with metal at the center of the ring include heme, (bacterio)chlorophyll, cobalamin, and factor F<sub>430</sub> with iron, magnesium, cobalt, and nickel inserted, respectively. Of these molecules, heme and chlorophyll are especially important to life and share a common biosynthetic pathway up to the protoporphyrin IX stage. Heme acts as an electron and oxygen carrier in most organisms. Chlorophyll is the primary molecule used to absorb light energy from the sun, thus initiating the process of photosynthesis. Class I chelatase enzymes play a critical role in tetrapyrrole factor biosynthesis, as they catalyze insertion of metal ions into the tetrapyrrole-derived rings. These enzymes share sequence and structural homology ([Brindley et al., 2003](#)); the three known class I chelatase enzymes are magnesium chelatase, cobalt chelatase and nickel chelatase, named after the inserted metal ion.

The enzyme magnesium chelatase from a purple photosynthetic bacterium, *Rhodospirillum rubrum*, is composed of three polypeptides: BchH, BchD, and BchI. A summary of the enzyme and the reaction features is presented in [Figure 1](#). The BchD and BchI polypeptides (and their homologs in the other chelatase enzymes) share significant homology with AAA+ proteins at the N terminus. AAA+ proteins are known to be ATPases and typically undergo large conformational changes as part of the catalytic mechanism. The AAA+ enzymes are sometimes referred to as mechanoenzymes as a result of the large structural changes observed to occur that are coupled to the production of mechanical force ([Glockner et al., 2000](#)). AAA+ enzymes typically form hexameric ring structures and can also form double ring structures where one of the rings does not have functional

ATPase activity. The BchD polypeptide also has significant homology to the integrin I domain and contains the MIDAS motif often found in integrin I domains and associated with Mg<sup>2+</sup>/Mn<sup>2+</sup> binding. [Lundqvist et al. \(2010\)](#) have used a combination of structural analysis tools to provide a coherent description of the conformational changes observed when the magnesium chelatase subunits with AAA+ homology are bound to ATP, ADP and the non-hydrolyzable ATP analog, AMPPNP.

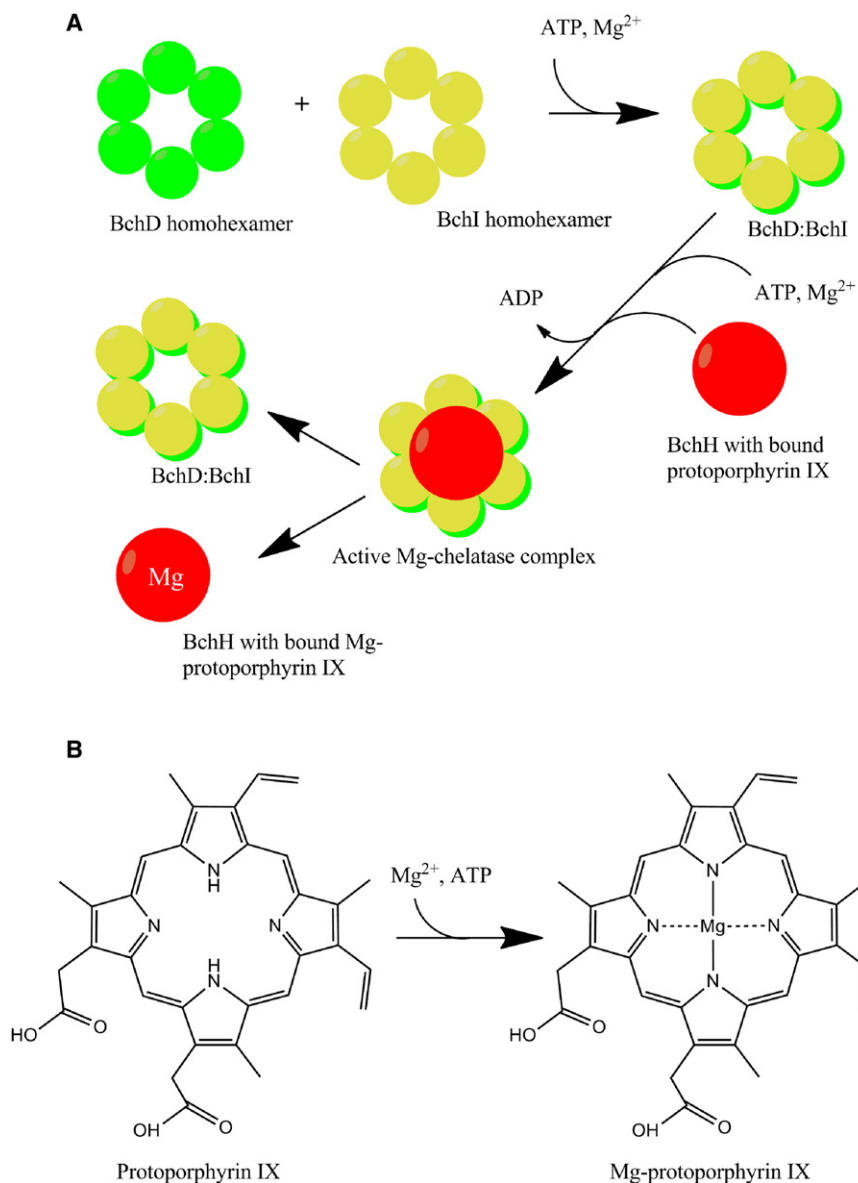
The authors used single particle cryo-EM reconstruction to determine the three-dimensional structures of the enzyme complexes with ATP, ADP, and AMPPNP. The reconstruction of the complex in the presence of ADP supported the previously reported double ring of hexamers ([Elmlund et al., 2008](#)). In the current reconstructions it is clear that the two rings are different in size. Based upon size of the subunits in question, the authors assigned the BchI polypeptides to the smaller ring and the BchD polypeptides to the larger ring. BchD:BchI complex formation is consistent with the kinetic analysis, suggesting that an ATP-dependent process of enzyme activation is required before the enzyme can begin to catalyze Mg-protoporphyrin IX synthesis (reviewed in [Willows and Kriegel \[2009\]](#)). Previous work also demonstrated that the BchD polypeptide is not capable of ATPase activity, but the BchI portion of the complex has an active ATPase.

Using the previously determined X-ray crystal structure of one of the subunits (BchI), the authors were able to create a homology model for BchD. Armed with both the structure of BchI and the homology model of BchD, [Lundqvist et al. \(2010\)](#) were able to model these polypeptides into the 7.5 Å resolution

reconstructions of the ADP-bound form of the complex. For both rings it appears that the basic unit is a dimer and each ring is a trimer of dimers. In a particularly satisfying extension of the modeling studies, reversible chemical crosslinking of the BchD:BchI complex was used to localize the BchI:BchD interactions. The observation that BchD monomers in a dimer are asymmetric, with one integrin I domain close to the BchI ring where the dimers interact and the other close to the BchI ring monomer-monomer interface is intriguing. It is also indicative of the role the integrin I domains may play in complex formation. The regions of BchI close to integrin I contact contain a motif known to interact with integrin I domains.

The significance of the integrin I domain interaction was further demonstrated by the comparison of the AAA+ chelatase unit bound to ADP with the ATP- and AMPPNP-bound complexes. Although the resolution of the reconstructions with AMPPNP- and ATP-bound complexes is lower than with ADP, the comparison suggests that there are significant changes in the structure of the complex when bound to the different forms of adenosine nucleotide. The AMPPNP-bound complex is more compact than the ADP-bound complex. Of particular note is the altered position of the integrin I domains of BchD that interact with the BchI protein. The integrin I domain close to the interface between the BchI homodimers has retracted and the other integrin I domain has moved closer to the BchI monomer-monomer interface, suggesting a role for integrin I domain movement in enzyme function.

The authors used interesting multivariate statistical approaches for the analysis of the ATP-bound complexes in response to the observed heterogeneity of this



**Figure 1. General Pathway for Catalysis by Magnesium Chelatase**

The steps in assembly of the magnesium chelatase are presented in (A). The BchD homohexamer forms a stable ring structure that interacts with BchI in the presence of ATP to form a double hexameric ring structure. This complex can then bind to the substrate, protoporphyrin IX, in a tight complex with BchH. The product released is Mg-protoporphyrin IX still bound to BchH.

(B) The structures of the magnesium chelatase substrate, protoporphyrin IX, and product, Mg-protoporphyrin IX.

complex. In this way, the authors were able to identify the major component that presumably represents the ATP-bound complex and use only those complexes in the majority state for further refinement of the reconstruction. Using this approach allowed them to reconstruct a complex very similar in structure to the ATP-bound complex found in the cobalt chelatase. The general trend of further compression

of the BchD:BchI complex is also observed. A fascinating discovery is that the ATP-bound complex has the BchI central cavity appearing closed off.

One of the remaining puzzles to be solved for the magnesium chelatase enzyme is how the BchH-protoporphyrin IX complex interacts with the BchD:BchI complex. Sirijovski et al. (2008) were able to reconstruct the BchH protein in

the presence and absence of protoporphyrin IX. It was clear in the work by Sirijovski et al. (2008) that a significant change occurred in the structure of BchH upon protoporphyrin IX binding, but the resolution of the reconstruction was 25 Å, insufficient to determine the location of protoporphyrin IX. At this point it is unclear if the BchH protein will dock on the BchD or the BchI ring, although the unusual features present in the BchI structure may suggest that docking occurs with that ring. Higher resolution structures of BchH and, more importantly, a trapped complex of BchH:BchD:BchI will be important to solving this puzzle.

An additional mystery is the location of the magnesium ion that is inserted into the porphyrin ring. The authors suggest that the MIDAS motif found in the integrin I domain of BchD could bind magnesium. The evidence for this magnesium being inserted is nonexistent, but an interesting speculation.

The structures presented do not provide a simple explanation for the dissonance between the three ATP molecules bound by the complex and the approximately 15 ATP used in catalysis. It is unlikely that the enzyme will ever be bound to all ATP or all ADP. As our understanding progresses, it is worth thinking of the BchD:BchI portion of the Mg-chelatase enzyme by analogy with ATP synthase having three different active sites at different stages in the catalytic process.

## REFERENCES

- Brindley, A., Raux, E., Leech, H., Schubert, H., and Warren, M. (2003). *J. Biol. Chem.* 278, 22388–22395.
- Elmlund, H., Lundqvist, J., Al-Karadaghi, S., Hansson, M., Hebert, H., and Lindahl, M. (2008). *J. Mol. Biol.* 375, 934–947.
- Glockner, G., Rosenthal, A., and Valentin, K. (2000). *J. Mol. Evol.* 51, 382–390.
- Lundqvist, J., Elmlund, H., Wulff, R.P., Berglund, L., Elmlund, D., Emanuelsson, C., Hebert, H., Willows, R.D., Hansson, M., Lindahl, M., and Al-Karadaghi, S. (2010). *Structure* 18, this issue, 354–365.
- Sirijovski, N., Lundqvist, J., Rosenbäck, M., Elmlund, H., Al-Karadaghi, S., Willows, R.D., and Hansson, M. (2008). *J. Biol. Chem.* 283, 11652–11660.
- Willows, R.D., and Kriegel, A.M. (2009). Biosynthesis of Bacteriochlorophyll in Purple Bacteria. In *The Purple Photosynthetic Bacteria*, C.N. Hunter, F. Daldal, M.C. Thurnauer, and J.T. Beatty, eds. (Dordrecht, The Netherlands: Springer Science), pp. 57–79.